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PHYTOPATHOLOGY

VOLUME X NUMBER 8

AUGUST, 1920

A PESTALOZZIA PRODUCING A TUMOR ON THE SAPODILLA TREE

NELLIE A. BROWN

WITH FIVE FIGURES IN THE TEXT

THE HOST

The sapodilla (*Achras zapota* Linn.) is one of the best indigenous fruits of the American tropics. The tree is cultivated in southern Florida, and grows wild on many of the Florida Keys. It is evergreen, with stiff glossy leaves, and a dense rounded crown, and reaches a height of 25 to 50 feet. The wood is hard and durable. The fruit has a variety of forms: round, conical, or globose depressed, 2 to $3\frac{1}{2}$ inches in diameter. The skin is thin and rusty brown in color, the flesh translucent, dark tan color, sweet, juicy, and delicious when well ripened. It is a great favorite in Florida, and is shipped successfully to New York City. In Florida this tree seems to be remarkably free from fungous diseases.

THE DISEASE AND ITS CAUSE

The disease reported in this paper occurred in Buena Vista, Dade County, Florida. It was found by Prof. P. H. Rolfs, Director of the Florida Experiment Station, who stated that the various tumors on the tree were from one year to eight years old. He considered the disease contagious but not enough so to infect surrounding trees, as only one other tree in the neighborhood had shown similar growths. The stem sent by him had a tumor 2 inches in diameter (fig. 1). This growth was rather smooth on the surface, which was partly covered with lichens and algae. There were also many tiny black pycnidia, but, like the lichens and algae, they were present on other parts of the stem and so had no significance. The gall appeared brown and dry but in cutting through, a soft portion was found outside the wood, which indicated that it was still alive. The

gall is of the hard type, composed mostly of wood, covered with an irregular layer of thickened bark. The cortex and the wood were examined carefully for bacteria or fungi, and a fungus was found penetrating the cells. No fruiting bodies were seen. Pieces of the gall were sterilized and placed in a moist chamber, and after a few days acervuli appeared containing Pestalozzia spores. Numerous isolations were made from the tumor by agar poured plates and sterile vegetable and agar tube cultures, and the fruiting bodies on both plate and tube cultures were those of a Pestalozzia.

FIG. 1. TUMOR OCCURRING ON THE SAPODILLA TREE IN AN ORCHARD AT BUENA VISTA, FLA.

SPORE CHARACTERISTICS

The conidia are four-septate with three central cells darker and a little larger than the hyaline cell at either end. Of the three central cells the lower one is usually lighter than the other two, and the central one darker

than the one above it (fig. 2a). In young cultures, but not too young, this feature is more pronounced than in the host material or in old cultures. The apical cell has three bristles which are quite constant; occasionally there will be four. The size of the spore is 16 to 24μ by 6 to 10μ. The bristle is 16 to 26μ. When the fungus is grown for four days on sterile potato cylinders, the size of the conidia is 20 to 26μ by 5 to 6μ. The bristle is 13 to 24μ. When grown for twenty days on cornmeal agar, the size of the conidia is 16 to 30μ by 6 to 8μ. The bristle is 16 to 26μ. Dozens of mounts of spores grown on different kinds of media were studied, and cultures of different ages. In all these only a few spores were

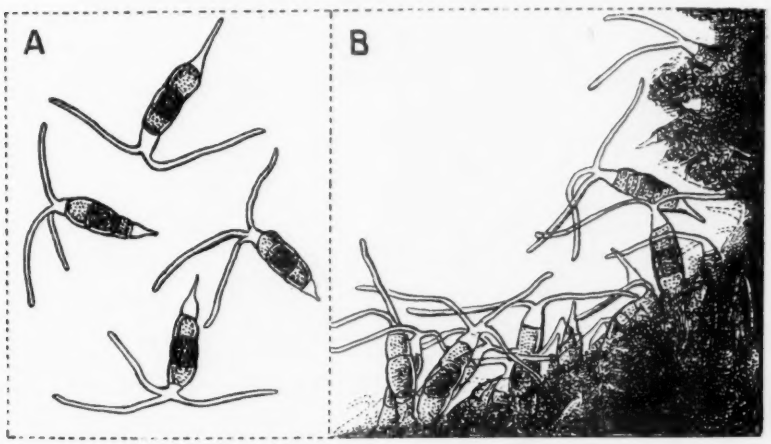

FIG. 2. *A*. Spores of Pestalozzia isolated from sapodilla tumor and grown on sterile potato cylinders. × 384.

B. Pestalozzia acervulus in mango tumor produced by the sapodilla Pestalozzia. × 384.

found which had five or six cross walls and an occasional one with seven or eight. The spores with six septa had six bristles, those with seven and eight had the usual number or none at all.

In old cultures, three months or older, spores with one dark cell and a hyaline end one with bristles sometimes occurred. No other form of fruiting body was seen.

Germination occurs usually from the lower of the three central cells, but it may occur from any other cell of the spore. Occasionally two germinating tubes are sent out from the same cell.

INOCULATIONS

It was necessary to have some sapodilla trees to inoculate, to prove the infectiousness of the strain isolated. The southern nurseries, however, either did not have them or did not think it advisable to ship so far north, and none could be procured. Later, fruit in the market at Miami was purchased by mail and sent to Washington and the seeds from them planted. The sapodilla is a slow grower and it was necessary to wait until the seedling trees were of a fair size to inoculate. This was about a year after the isolations were made. In a month and a half small outgrowths appeared at the inoculated places on about 75 per cent of the stems. These increased very slowly in size, corresponding with the slow growth of the trees (figs. 3 and 4). The inoculations were made by smearing the spores on the stems and then puncturing with a sterile needle. The trees were kept in a greenhouse where the temperature was usually 75° to 80° F.

FIG. 3. A YOUNG SAPODILLA TREE WITH TUMORS PRODUCED BY INOCULATING WITH THE SAPODILLA PESTALOZZIA NOVEMBER 11, 1915

Photographed March 14, 1916

Two isolations were tested out, one which produced an abundance of spores, the other at first a meager amount of spores. This latter isolation proved to be the more infectious. One thing of interest was noted during the inoculation work; the very youngest and most tender tissue did not become infected as readily as the older tissue. The stems, however, were all comparatively young and not as yet toughened, but the stems at the growing point did

not produce outgrowths. However, reddish brown spotting of very young leaves of the sapodilla occurred, while the toughened leaves remained unchanged when inoculated.

When the galls were about a centimeter in diameter the fungus was reisolated and other small sapodilla trees inoculated with the reisolation colonies. In due time galls formed on these trees also, but only about half of the inoculations proved infectious.

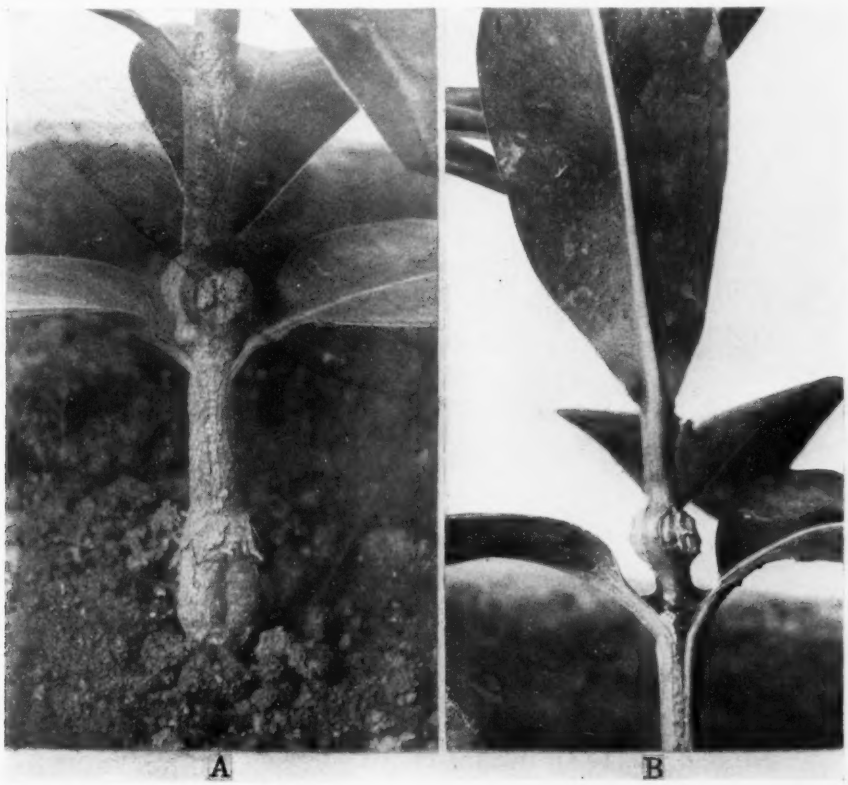

FIG. 4. *A*. Young Sapodilla tree with tumor produced by inoculating with the sapodilla Pestalozzia November 11, 1915 Photographed July 13, 1916.

B. Young sapodilla tree with tumor produced by inoculating with the sapodilla Pestalozzia November 11, 1915. Photographed March 1, 1916.

INOCULATIONS INTO OTHER PLANTS

Inoculations were also made into other plants and trees, some of which are susceptible to the crown gall organism, *Bacterium tumefaciens*. Rose bushes, sugar beets, Paris daisy, citrus trees, pelargonium, avocado, be-

gonia, cacao, allepo pine, white pine, spruce, hemlock, larch, balsam, olive, and mango were inoculated, several tests being made with some of them.

No outgrowths formed on any except the mango, *Mangifera indica* Linn., olive, *Olea europaea* Linn., and balsam, *Abies balsamea* Linn. Small galls, 3 to 10 mm. in diameter, formed on the olive and balsam in the course of a year, and large galls, 1 inch in diameter, on the mango. In mangoes grown in greenhouses swellings at nodes frequently occur. These nodal swellings need not be confused with the tumors produced by the fungus, as they are easily distinguished.

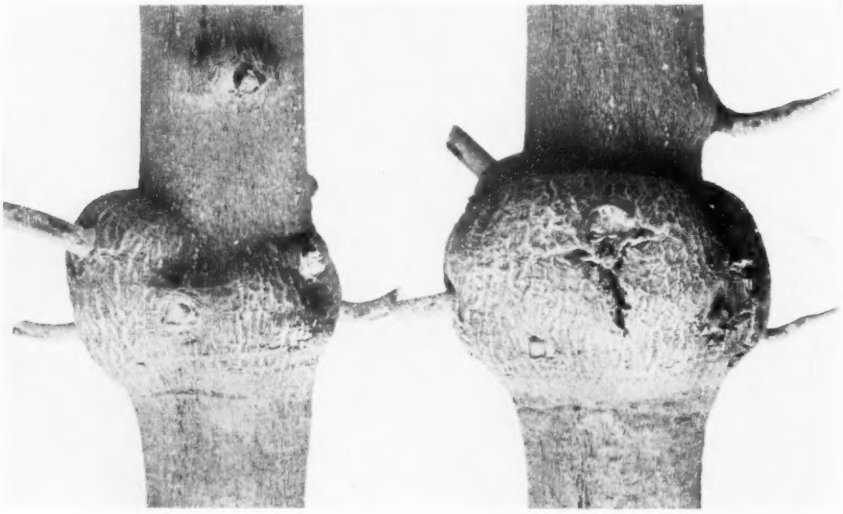

FIG. 5. TUMOR ON MANGO TREE

Tree inoculated July 10, 1916, with sapodilla Pestalozzia. Photographed June 28, 1918.

The tumor on mango grew very slowly, in fact, no definite outgrowth had taken place in three months. In six months there was a typical tumor encircling the stem as is the case in the sapodilla. This growth continued slowly for two years, when it had reached a diameter of 2 inches (fig. 5).

One of the mango tumors was cut off three years after the tree was inoculated, part of it placed in a moist chamber, and the other part used successfully for reisolating the fungus. In a few days the portion in the moist chamber showed the acervuli breaking through the surface of the gall and the typical Pestalozzia spores appearing (fig. 2, B). The fungus was also isolated from the olive gall.

INOCULATIONS INTO CONIFERS

The fungus was also infectious to the larch (*Larix occidentalis* Nutt.), blue spruce (*Picea pungens* Englm.), and hemlock (*Tsùga canadénsis* Sarg.), producing a blight instead of outgrowths. The blighting occurred in young leaves when the spores were sprayed on them. No wounding was necessary. This condition was carried to other young leaves as they continued to appear during the summer. The check plants were not infected. Several attempts were made to infect the white pine in the same way, with negative results. The trees used were young, none of them being over 16 inches tall. They had been kept in pots out of doors during the winter, then in early spring brought into the greenhouse and repotted. At the time of inoculation, young leaves were growing freely. After inoculation, some of the trees were put in an infection cage while others were kept in the same greenhouse in the open. This was done to see if the excessive moisture of the cage would favor infection. The temperature of the greenhouse ranged from 72° to 78° F.

The trees were inoculated in two ways: in one case the spores were washed off the cultures into sterile water and the plants sprayed with an atomizer, some of them being wounded by needle pricks; in the other the spores were smeared on the stems and leaves and wounds made by puncturing with a delicate sterile needle.

No galls were produced on any of the trees, but a very decided blight occurred in the young larch leaves, a fair amount of blight in the young leaves of blue spruce, and a trace on the hemlock. The inoculations of these three were as follows:

Two larch trees 16 inches tall were inoculated, one being sprayed and wounded, the other sprayed only. Both were kept in an infection cage for five days. Blighting of the leaves occurred in both trees two weeks after they were taken from the infection cage. The check in the open remained healthy. Razor sections showed fungus mycelium penetrating the cells, and when blighted leaves were placed in a moist chamber, the Pestalozzia spores were produced in abundance. This blighting continued as other young leaves pushed out during the summer. The wounded tree was affected somewhat more than the other.

Three blue spruces were inoculated. Two were sprayed with spores without wounding, then placed in an infection cage; the other tree was smeared with spores and punctured, but not placed in a cage. Of the two sprayed, only one became infected. The one punctured and not kept in the cage became infected slightly more than the other. Evidently the conditions of moisture both in and out of the infection cage were sufficient to infect, and the wounding helped to increase infection.

Only one hemlock tree 12 inches tall was available; it was sprayed but not punctured, then kept in the infection cage for five days. In two weeks there was slight blighting but this did not continue to advance.

The inoculations into the conifers were made because of the infectious character of *Pestalozzia funerea* to white pine (*Pinus strobus* L.), Norway spruce (*Picea excelsa* Link), and hemlock (*Tsùga canadénsis* L.), as demonstrated in the work of John J. Wenner (13), in which he shows that seedlings one month old to three-year-old trees under certain conditions of moisture are blighted by this species, and because *Pestalozzia hartigii* has been recognized as the cause of a seedling disease of the spruce and the silver fir in Europe (1), (3), (11), (12).

The balsams (*Abies balsàmea* L.) were inoculated more especially because of a tumor reported by P. Hennings (5) on *Abies balsàmea*, produced by a Pestalozzia which he has named *Pestalozzia tumefaciens*. The galls resulting from needle-prick inoculations into balsam with the sapodilla isolation were very slow-growing and small, the largest one reaching hardly more than a centimeter in diameter in a year and a half. The size is about the same or a little larger than those in the Pathological Collection of the United States Department of Agriculture, produced by *Pestalozzia tumefaciens* (P. Hennings). However, in his description of this particular out-growth which appears in different species of Abies, Hennings states that they reach a size of from 1 to 5 centimeters in diameter, and a badly attacked tree must be cut out and burned in order to check the spread of the disease.

COMPARISON OF THE SAPODILLA PESTALOZZIA WITH OTHER TUMOR-FORMING PESTALOZZIAS

The spores of *Pestalozzia tumefaciens* do not agree in size and number of cells with the Pestalozzia isolated from the sapodilla. There are other differences, e.g., in color of central cells and shape of end cells. *Pestalozzia tumefaciens* is 13 to 17μ long by 5 to 6μ wide, bristle 25μ. Spores are cylindrical-oblong with three cross walls, making 4 cells. The two middle cells are large and dark brown in color, while those at each end are colorless and somewhat papillary. The upper cell bears three very fine, spreading, colorless bristles.

Nor do the spores or the sapodilla Pestalozzia agree with those of *Pestalozzia gongrogina* (F. Temme) (10), which according to Temme produces a gall on *Salix viminalis*. These willow galls grow from year to year, the largest ones becoming the size of a hen's egg. They are soft and green at first and later become woody; in the course of a few years the trees are covered with various sizes of galls. This description is not

unlike that of the galls on willow due to the crown-gall organism, *Bacterium tumefaciens*. P. Hennings became interested in the tumors on *Salix viminalis*, and found that the disease was infectious to other varieties of willow. Neither Hennings nor Temme produced galls by inoculations with pure cultures of *Pestalozzia tumefaciens* or *Pestalozzia gongrogina*. In his description of the latter, F. Temme states that spores are produced in a definite pycnidium. They are slightly bent, club-shaped, 0.024 mm. long by 0.004 mm. wide, 3-celled, colorless, with a delicate curved bristle at upper end.

Mr. I. B. Pole Evans and Miss Ethel M. Doidge (2) of the Transvaal Department of Agriculture found a Pestalozzia on *Cydonia vulgaris*. Warty outgrowths formed by larger numbers of arrested adventitious roots, they claimed, were produced by this fungus. As the spore has but one bristle it is unlike that of the Pestalozzia considered in this paper. Evans and Doidge have named their fungus *Pestalozzia* (Monochaetia Sacc.) *cydoniae*.

ANOTHER PESTALOZZIA ON A RELATED HOST

There is a *Pestalozzia sapotae* (P. Henn.) n. sp. reported from Brazil on the leaves of *Achrae sapotae* (4). The spots are dark, wide spreading, with acervuli on the upper side of leaf bursting out black. Conidia spindle shaped, 4-septate; three cells black, 10 to 14μ by 5 to 6μ, end cells hyaline; papillate, three filiform bristles 10 to 15μ, pedicel short and hyaline.

As there has been confusion regarding the botanical name of the sapodilla, the *Achrae sapotae* mentioned above may or may not be identical with *Achras zapota*, the tree on which the tumor occurred. The description of the Pestalozzia producing the spotting of the leaves does not correspond to that of the Pestalozzia producing the tumors on *Achras zapota*.

Leaves of sapodilla inoculated with the sapodilla tumor Pestalozzia produce rather reddish, well defined spots, and the conidia in these spots maintain the typical characteristics of those produced in the tumor.

As there are essential differences between the spores of the Pestalozzia forming the tumor on the sapodilla and those forming tumors on the other plants, namely, balsam and willow, the tumor-forming Pestalozzia of sapodilla cannot be classed with either of them. It resembles *Pestalozzia funerea* in some respects (8), i.e., the spores are very much like the funerea spores, it is pathogenic, and it also produces a blight on some of the members of the pine family. However, *Pestalozzia funerea* is not known to produce tumors. Comparison was made with spores of *Pestalozzia guepini* Desm., a species known to be parasitic, but there were well defined differences between the two (9). The descriptions of other species reported

to be parasitic were studied, but no agreement was found between them and that of the sapodilla species (6) (7). It seems best, therefore, to consider this fungus as a separate species, and because of its tendency to produce hard galls, the name *Pestalozzia scirrofaciens* is suggested and the following characterization given:

Pestalozzia scirrofaciens n. sp.

The acervuli are immersed, breaking out to the surface and of a black color after the gall has been kept in a moist chamber a few days. The conidia are ovate-oblong to spindle shape, slightly curved, 4-septate with slight or no constriction of the septa. The three central cells are olive brown, the terminal cells colorless, conoid, bearing at the apex three, rarely four, slender bristles bent outward, the pedicle is short and slender. Size of spores 16 to 24μ by 6 to 10μ. Bristle 16 to 26μ.

Habitat. In stems of *Achras zapota*, Florida, producing a hard tumor.

FAVORABLE MEDIA FOR GROWTH OF THE FUNGUS

Sterile potato cylinders are a very good medium for the growth of this species, and the fungus will continue to live thereon over a year and a half but does not live two years, temperatures being 12° to 17°C. Carrot and potato agar are also good. There is very little mycelium produced on cornmeal agar, but it is luxuriant on potato cylinders and sugar-beet agar. Acervuli are produced in abundance on plain potato cylinders, and occasionally black spore masses protrude in a horn like shape. Weak acids such as 1 and 2 per cent malic acid added to potato cylinders retard the growth of the mycelium and lessen the production of spores.

In three to seven days after transferring to potato cylinders, small, black dots appear on the surface, and later black, liquid-like drops exude from them. One of these drops examined under the microscope was found to be made up of masses of conidia. When young, these small, black bodies have a structure which to all appearances is a pycnidium. As development goes on, however, they break open across the top and we have acervuli extruding spores.

CONTROL

The control method which proved successful in the Buena Vista orchard where the disease was found is the one to be recommended; namely, cutting down and burning the infected trees. This method was used successfully with trees infected by other gall-producing Pestalozzias.

SUMMARY

Tumofs of various sizes occurred on stems of the sapodilla tree growing in an orchard in Dade County, Florida.

A Pestalozzia was isolated from one of these outgrowths, and inoculations into young sapodilla trees produced slow growing tumors from which the fungus was reisolated.

Small galls were produced on the balsam fir and olive, and large ones on the mango tree.

Inoculations into larch, hemlock, and blue spruce produced blighting of the leaves but no tumors. No blighting occurred on the white pine.

The Pestalozzia isolated from the sapodilla tumors does not agree with *Pestalozzia tumefaciens*, which produces tumors on different species of Abies. Neither does it agree with the Pestalozzia reported to produce tumors on different species of Salix.

It agrees in some respects with *Pestalozzia funerea* Desm., which is a parasitic species but one not known to produce galls.

The name *Pestalozzia scirrofaciens* is suggested.

The disease can be controlled in an orchard by destroying the infected trees.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

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BRIEFER ARTICLES

NOTES ON PERIDERMIUM HARKNESSII

GEORGE G. HEDGCOCK AND N. REX HUNT

Peridermium harknessii Am. Auct. as used in this paper applies to the gall-producing form of Peridermium which is the aecial stage of *Cronartium coleosporoides* (Diet. & Holw.) Arthur. This species attacks both *Pinus contorta* and *Pinus ponderosa* in some regions in the western and northwestern states, more especially the former species of pine in Colorado and Wyoming. The galls produced by this species vary in shape from fusiform swellings on recently infected small twigs to sub-spherical galls from older infections on limbs, twigs, and small trunks which are encircled by the diseased tissues. Infections on one side of a trunk or limb may produce a hemispherical form. Young trees often are either killed or stunted by the strangulating effect of these galls.

Rodents, especially squirrels and porcupines, feed upon the cambial layer of the galls of *Peridermium harknessii*, owing to the sweet taste of this layer when either aecia or pycnia are present or in process of formation. The senior writer has repeatedly observed them feeding near Fox Park, Wyoming, on galls of *Pinus contorta*. On a gall defaced by these rodents, the wood dries out and the bark is not replaced. Frequently new gall tissue forms in a fold adjacent to the wound, producing an appearance commonly called "cat-face" in this region. In a timbered area being cut over near Fox Park, it was noted that both *Trametes pini* (Brot.) Fr. and *Polyporus schweinitzii* Fr. frequently gain entrance through such wounds.

The action of these rodents in feeding not only on the galls of this Peridermium, but also on the healthy wood of adjacent small trees, suggested to the writers the possibility of aecial infection taking place through their depredations. This was strengthened by observations on the spread of the Peridermium in some localities in this region in the apparent absence of telial hosts beneath the trees.

On April 20, 1912, six trees of *Pinus sabiniana* were inoculated by inserting in a wound under the bark a small piece from the cambium of the immature fruiting portion of a live gall of *Peridermium harknessii* on *Pinus attenuata* collected by Dr. E. P. Meinecke in California, April 12, 1912. The wounds were tightly wrapped with wet cotton. Six control

trees were similarly wounded and wrapped. Two out of the six trees inoculated developed typical galls of *Peridermium harknessii* from these graft inoculations but died before aecia were produced.

On June 26, 1915, forty-four trees of eleven species of pine were inoculated as follows: Fruiting galls of *Peridermium harknessii* from *Pinus contorta*, collected by Ellsworth Bethel at Tolland, Colorado, June 20, were held over a sheet of paper and the aeciospores dislodged by jarring the galls. Wounds were made by inserting a scalpel under the cambium of the pine trees to be inoculated, then spores were introduced in the wounds, which were then tightly wrapped with strips of wet cotton.

Of the trees inoculated, sixteen, or 36 per cent, became infected at the point of inoculation. Oblong galls were formed. Of these infected trees, more than half have since died from the effects of the disease. Four trees, all of *Pinus ponderosa*, produced aecia on the galls, three in 1917, and one in 1918. No pycnia were observed.

At the time of inoculation aeciospores taken from those used in the experiment were germinated on the surface of sugar agar poured plates. Of the spores that germinated, all produced the usual aecial mycelium, and none the promycelium characteristic of teliospores.

The number of trees inoculated of each species, and the number infected was as follows: *Pinus caribaea*, six inoculated and three infected; *P. contorta*, six and four; *P. halepensis*, one and one; *P. mayriana*, six and two; *P. palustris*, two and none; *P. pinea*, one and one; *P. ponderosa*, two and none; *P. ponderosa scopulorum*, nine and four; *P. sabiniana*, two and one; *P. taeda*, six and none; and *P. virginiana*, three and none.

Two other similar experiments have been performed, one with aeciospores of *Peridermium harknessii* from *Pinus ponderosa*. A few galls were obtained, mostly on *Pinus ponderosa*, but none fruited. One tree of *Pinus virginiana* was infected, which is an additional species to those named above.

These experiments were all performed with potted plants grown from the seed under controlled conditions in the green-houses at Washington, D. C., and apart from other experiments with gall-forming species of Peridermium. Control trees of each species of pine used remained free from infection. The telial stage of *Cronartium coleosporoides* has never been found in Colorado and Wyoming, antedating or coincident with the aecial. This precludes a possibility of the presence of sporidia on the galls.

Peridermium harknessii has been collected in nature on the following species of pine in the United States, the data being taken from collections in this office at Washington, D. C.: On *Pinus attenuata* Lemm., two collections from California; on *P. contorta* Loud., two from California, fifty-

one from Colorado, eight from Idaho, eight from Montana, six from Oregon, one from South Dakota, one from Washington, and twelve from Wyoming; on *P. jeffreyi* "Oreg. Comm.," two from California; on *P. ponderosa* Laws., three from California, four from Idaho, and two from Montana; on *P. ponderosa scopulorum* Engelm., six from Colorado, one from Nebraska, and three from South Dakota.

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PYTHIACYSTIS AND PHYTOPHTHORA[1]

H. S. FAWCETT

The following brief discussion is given here in order to correct, as far as possible, any confusion in regard to the distribution of *Pythiacystis citrophthora* that the author's previous publications may have left. The writer has previously reported finding on Citrus, in Florida, Cuba and the Isle of Pines, a fungus that he then considered to be *Pythiacystis citrophthora* (1). The cultures on which this report was based, though differing somewhat from those of *Pythiacystis citrophthora* Sm. and Sm. found in connection with lemon brown rot (2) and in connection with one type of gummosis (3), were thought to be sufficiently similar to be considered a variety or strain of the above mentioned species. The sporangia and zoospores and also the mycelial growth at certain temperatures were similar in appearance to *P. citrophthora*. There appeared also a close similarity in their behavior as compared to *P. citrophthora*, when inoculated into citrus bark. Later it was found that the growth temperature relations were markedly different from that of *P. citrophthora* (4). The behavior of the Florida-Cuba cultures in the rotting of fruit was also seen to be slightly different from that of *Pythiacystis citrophthora* in California, and yet the similarity in the appearance of the rot produced was more striking than were the differences.

In order to correct any misunderstanding that may have arisen as to the identity of the fungus reported upon in the first mentioned publication, the writer wishes to state that the cultures which he found in connection with mal di gomma in Florida, Cuba and the Isle of Pines were all apparently *Phytophthora terrestria* Sherbakoff (5), and not typical *Phythiacystis citrophthora*. Sherbakoff has kindly examined those from Florida and Cuba, and considers them to be identical with his fungus. Stevens

[1] Paper No. 69, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

also (6) has made an extensive cultural examination of diseased bark in connection with mal di gomma in various parts of Florida, and has repeatedly isolated a fungus which appears to be identical with *P. terrestria*. It may also be of interest here to state that a culture which appears to be identical with *P. terrestria* was isolated by the writer from diseased bark at the base of a young orange tree from Lindsay, California. This, so far as is known, is the only time this form has been obtained from California specimens. Most of the California strains obtained from fruits and diseased bark of citrus do not readily show the perfect stage in cultures, and are like *Phythiacystis citrophthora* as described by Smith and his co-workers. Cultures obtained from Arizona specimens of young orange trees killed by gummosis were of the *Phythiacystis citrophthora* type. A Phythiacystis-like fungus from citrus received by the writer from George Fawcett from Tucuman, Argentine Republic, when submitted to Sherbakoff was thought by him to be nearly if not entirely identical with *Phytophthora terrestria*.

A number of different Phythiacystis-like forms have been found in California on other hosts. Barrett (7) has reported on some of these and has suggested that some of them, at least, are probably true Phytophthora. *Pythiacystis citrophthora* itself has so many cultural characteristics in common with known Phytophthora, such as *P. terrestria*, for example, that one is led to suspect that if its perfect stage were known it would prove to be closely related to the above species.

The author (8) also reported a Pythiacystis-like fungus affecting avocado trees. A further study of this culture indicates that it is probably a Phytophthora, and quite different in some of its cultural characteristics from either *Pythiacystis citrophthora* as here considered, or *Phytophthora terrestria*.

To summarize, most of the cultures so far made from fruit and from the diseased bark of one type of gummosis of citrus in California appear to be *Pythiacystis citrophthora* proper as described by Smith & Smith. In one instance only has *Phytophthora terrestria* been found on citrus in California. Cultures made from diseased bark of mal di gomma (footrot) specimens of citrus in Florida, Cuba, the Isle of Pines and the Argentine Republic all appear to have been *Phytophthora terrestria* as described by Sherbakoff. Other Pythiacystis-like forms so far obtained from hosts other than citrus in California appear to be different from either of the above species.

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SOME METHODS FOR INVESTIGATING INTERNAL SEED INFECTION

J. B. S. NORTON AND C. C. CHEN

The technique for the study of diseases carried by internal seed infection has never been standardized, for the methods are still in the experimental stage and no extensive or systematic investigation of seed diseases has yet been made. However, a good general technique will be needed when such studies are made. In the course of investigations on internal seed diseases, the writers have developed a technique that gives rather successful and practical results. This has been developed from the method used by Selby and Manns[1] in their studies on diseases of cereals and grasses. Our method is outlined as follows:

1. Soak the seeds to be studied in water from ten to twelve hours. The presoaking is to render the spores of pathogens on the seed coats more susceptible to the action of the disinfectant, and at the same time to prevent seed injury by the disinfectant. The efficiency of this treatment has been tested out also by Braun.[2]

2. Shake the presoaked seeds from three to five minutes in an alcoholic solution of corrosive sublimate (2 grams to 1000 cc. of 50 per cent alcohol) in a sterilized test tube.

3. Remove the traces of the disinfectant from the seeds by first washing once in ethyl alcohol (93 per cent) and then three times in sterile water. The writers have determined by a series of experiments that the latter operation is absolutely necessary in order to prevent the alcohol

[1] Selby, A. D., and Manns, T. F. Studies in diseases of cereals and grasses. Ohio Agr. Expt. Sta. Bul. 203: 213–218. 1909.

[2] Braun, H. Presoaking as a means of preventing seed injury due to disinfectants and of increasing germicidal efficiency. Science, n.s., **49**: 544–545. 1919.

from injuring the seeds. In the tests summarized in the following table, one hundred seeds of each kind, presoaked in water for ten hours, were used for each trial. They were given the standard germination test in the State Seed Laboratory. The table shows the details and results.

TREATMENT	PERCENTAGE OF GERMINATION			
	Wheat	Corn	Peas	Soy-beans
No treatment	72.5	88.0	92	92.5
Immersed in corrosive sublimate (alcoholic) solution for five minutes	0	74.5	58	89.0
Immersed in corrosive sublimate (alcoholic) solution for five minutes and washed in 93 per cent alcohol	0	20.0	11	53.0
Immersed in corrosive sublimate (alcoholic) solution for five minutes; then washed in 93 per cent alcohol; then washed in water three times	69.5	91.0	77	90.5

The percentages in the table represent the average of two duplicate tests for each kind of seed.

Probably the alcohol washing could be eliminated, but it is desirable to remove any chance of interference with the growth of internal organisms.

4. Pick out the surface-sterilized seeds with sterile forceps and plant them in sterile nutrient agar plates for germination, during which many of the pathogens carried internally will develop sufficiently to be recognized and can then be isolated for further examination.

Or, plant the surface sterilized seeds in large, thick test tubes containing absorbent cotton saturated with a sterile nutrient solution. This practice enables the investigator to discover the presence of parasites carried internally by the seed, and to actually determine their effect upon the young plant. The nutrient solution enables the seedlings to continue their growth, so that death from lack of nourishment may not be confused with the action of parasites. The cultures may be placed in a moist chamber to aid germination.

5. Make pure cultures from the organisms developing from the infected seeds, and inoculate healthy plants with the isolated organisms both in tube cultures and in pot cultures in sand.

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REVIEWS

A noteworthy contribution to the literature on tropical plant diseases is "The Red Ring or 'Root' Disease of Coconut Palms," by William Nowell, published in the W. I. Bul., vol. xvii, no. 4, 1919 (see also Agric. News, p. 398, December 14, 1918, and Agric. News, p. 46, February 8, 1919).

Up to the time of Nowell's work on this disease it had been reported many times but usually referred to as root disease, merely intending to indicate that, supposedly, the trouble arose in the roots or in the soil. The history of the disease is given, and its distribution as found by Nowell in Trinidad and Tobago. To this might be added Panama and Cuba as observed by the reviewer.

The external symptoms are a progressive yellowing and browning of the leaves from below upwards, commencing at the tip of each leaf. The internal symptoms consist of a red zone in the stem, which is some distance from the bark and, at least at the base, forms a complete circle.

The red tissues are reported always to be heavily infested with nematodes which Cobb has described as a new species, *Aphelenchus cocophilus*. This fact and the fact that much careful work has failed to demonstrate the presence of either bacteria or fungi have led to the conclusion that the nematode is the cause of the trouble.

This disease has been and is serious in certain places, destroying great numbers of trees, usually about the age when they come into bearing.

No specific remedy is proposed, but experiments are being conducted by Nowell looking to the control of this pest. At least a great advance has been made in demonstrating the presence of a pest in the diseased tissues.

Nowell's theory as to a possible manner of infestation is of interest and warrants further investigation. The theory is that the nematodes may occur in the seed bed, there infest the young plant about the base of the leaves, and from that point work downward as the trunk develops. This is borne out by the fact that occasionally red ring is found in the trunk when the roots are free from the pest.

JOHN R. JOHNSTON.

PHYTOPATHOLOGICAL NOTES

Personals. Dr. Edgar W. Olive has resigned as Curator in the Brooklyn Botanic Garden. He has gone into private business, his address being 904 Hume-Mansur Building, Indianapolis, Ind.

Dr. George L. Peltier has given up his position at the Alabama Agricultural Experiment Station and accepted an appointment as pathologist at the Nebraska Agricultural Experiment Station.

Dr. G. R. Bisby, assistant pathologist at the University of Minnesota, has been elected pathologist in the Manitoba Agricultural College at Winnipeg. Dr. Bisby assumed his new duties June 1.

Mr. A. F. Thiel, who has been stationed at St. Paul, Minnesota, on rust epidemiology studies, was elected assistant pathologist at the Alabama Polytechnic Institute, Auburn, Alabama. Mr. Thiel began his duties at Alabama on April 1.

Dr. Julian G. Leach, associate professor of botany in the Colorado Agricultural College, has been appointed assistant pathologist at the University of Minnesota.

Professor Frank T. McFarland, University of Kentucky, has been granted a year's leave of absence. He has taken up graduate work in the Department of Plant Pathology of the University of Wisconsin.

LITERATURE ON PLANT DISEASES[1]

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[1] This list includes references, both American and foreign, to the literature of plant pathology and mycology of interest to pathologists. Foreign references published since January 1, 1917, have been included beginning with the list appearing in v. 7, no. 3, June, 1917.

All authors are urged to cooperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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